A RESEARCH PROPOSAL

DESIGN, DEVELOPMENT AND EVALUATION OF BIOACTIVE LOADED METAL OXIDE NANOMATERIAL FOR TARGETED DRUG DELIVERY

Submitted

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INTRODUCTION

The development of nano-sized materials with distinctive physical and chemical properties that make them suitable candidates for biomedical applications is made possible by advancements in nanotechnology and interdisciplinary research. In general, nanotechnology entails the synthesis and manipulation of matter at scales of a few hundred nanometers to enable particular properties that depend on scale [1]. The preferred size for nanoparticles (NPs) used in nano-medicine should be less than 200 nm [2]. The ability of NPs to cross the blood-brain barrier, enter the pulmonary system, and adsorb through endothelial cells is demonstrated by their small size and large surface area, which exhibit enhanced colloidal stability and, consequently, increased bioavailability [3]. Metal oxide nanoparticles (MONPs) in particular have some benefits, including high stability, straight forward preparation procedures, easy engineering to the desired size, shape, and porosity, no swelling variations, easy incorporation into hydrophobic and hydrophilic systems, and easy functionalization by various molecules due to the surface's negative charge [4]. These features make MONPs a promising tool for biomedical applications. It is important to define the morphology of MONPs because different in vivo systems react to them differently depending on their size, shape, purity, stability, and surface characteristics. As shown in Figure 1, MONPs can be divided into zero-, one-, two-, and three-dimensional categories [5] based on the number of dimensions, which are not limited to the nanoscale. Nanoparticles (NPs), nanoclusters, quantum dots, and other materials with all dimensions smaller than a nanometer are examples of zero-dimensional nanomaterials. One dimension is outside the nanoscale for one-dimensional NPs like nanorods, nanotubes, nanowires, and nanofibers. Nanosheets, nanocoatings, nanofilms, and nanoplatelets are examples of two-dimensional or planar nanomaterials that have two dimensions outside of the nanoscale. Three-dimensional NPs include dendrimers, bundles of nanowires or nanotubes, nanopillars, nanoflowers, multi-nanolayers, and other structures. They develop as a result of the agglomeration of nanomaterials at a size greater than 100 nm in all three orthogonal directions. Numerous techniques have been developed for the synthesis of these MONPs, which are outlined in earlier reviews [6]. MONPs can be organized with crystal morphologies that exhibit a variety of reactive sites and corners and are typically highly ionic in nature. The realization of nanodevices for various applications in chemistry, electronics, optics, and biomedicine has a promising potential to be achieved through the deposition of MONPs on the desired position with a nanoscale resolution on a specific substrate [7].

In recent years, the biomedical use of NPs for therapeutics and diagnostics (drug delivery and improved performance of medical devices) has advanced quickly. There are numerous benefits to using MONPs in modern medicine for both diagnosis and treatment. The engineering of water-dispersible NPs enables the use of these particles in a wide range of fundamental or applied biomedical studies. NPs are currently utilized in diagnostic imaging for a variety of molecular markers, genetic and autoimmune diseases, cancerous tumors, photosensitizers in photodynamic therapy, and target drug delivery [8]. Today, NPs are used in biomedicine as diagnostic imaging materials, such as iron nanooxides for magnetic resonance imaging and functional fluorescent-labeled particles (semiconductor quantum dots) for fluorescent imaging. By summarizing fresh ideas, taking advantage of MONPs' benefits, and highlighting successful studies of MONP applications in nanomedicine, this work aimed to explore the difficulties that would motivate further research. An emphasis has been placed on the antibacterial properties of greenly synthesized nano-oxides as well as the toxicities of MONPs to living things.

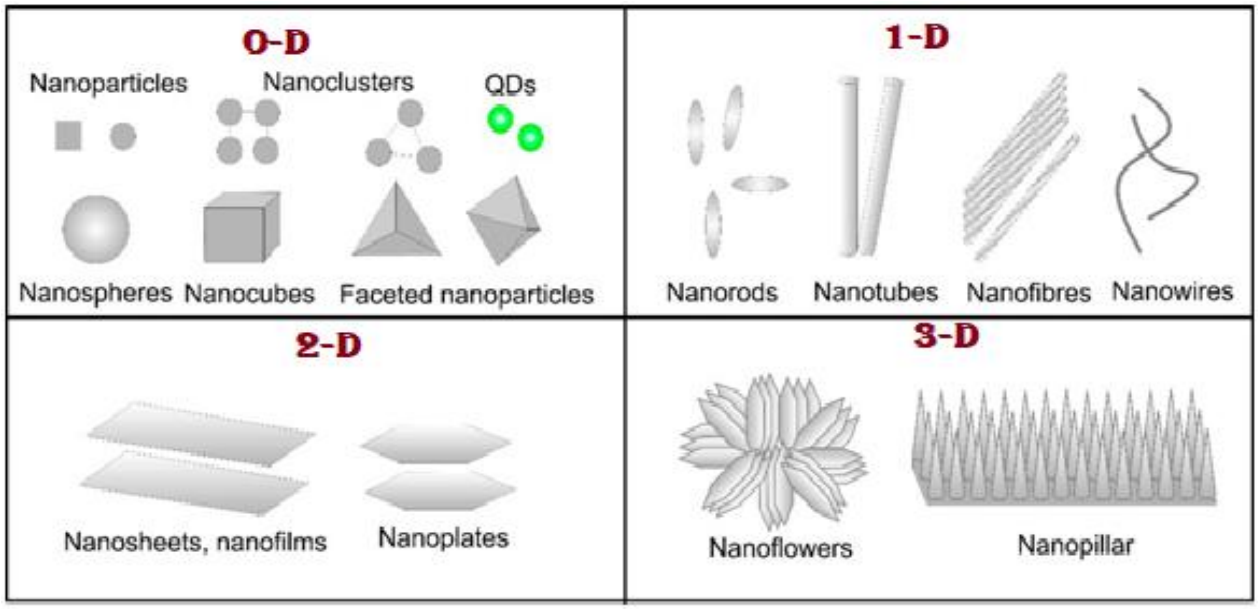

Figure 1. Scheme of zero-, one-, two- and three-dimensional nano-structured materials with different morphologies 0-D, 1-D, 2-D, and 3-D indicate zero-, one-, two- and three-dimensional NPs, respectively.

Physical-Chemical Properties of Nano-Oxides

When NPs are ingested into the body, they interact with cell biomolecules and biofluids, which makes it easier for the particles to physically move into the inner cellular structures [9]. Controlled synthesis techniques are concentrated on producing NPs with tailored morphological configurations, sizes, distributions, and stability because the biological response to NPs depends on a variety of factors, including size, morphology, aggregation, and so forth. Here is a list of the key factors that affect how well NPs perform when they come into contact with cells:

Shape and Size

The surface-to-volume ratio is determined by size and has an impact on biodistribution and material uptake [10]. Elsabahy and Wooley claim that the intermediate size (20-100 nm) of NPs has the highest potential for in vivo application due to the ability to propose enough circulation time because the openings of the blood vessels in many tumors are less than 200 nm and the mammalian vasculature has a pore size of about 5 nm and below this size [11]. According to studies, iron oxide nanoparticles (NPs) with a diameter larger than 100 nm were quickly taken up by macrophages and eliminated through renal clearance, while NPs with a diameter smaller than 10 nm were quickly taken up by the liver and spleen [12]. Numerous studies demonstrated that smaller than 5 nm NPs could cross cell barriers through translocation or other non-specific mechanisms, whereas larger particles needed specific or non-specific cell transport mechanisms to enter the cell, such as phagocytosis or pinocytosis [8]. Nanosized oxides have been created in a variety of shapes, including nanorods, nanospheres, nanocubes, nanowires, nanotubes, and more [13]. According to Andrade et al. [14], the larger area of contact between the rod-shaped NP and the cell membrane explains why rod-shaped NPs are more successfully absorbed by cells than spherical NPs. Tripod iron oxide NPs were found to be less toxic to HeLa and Hepa 1-6 cells than spherical iron oxide NPs at concentrations between 0.022 and 0.35 mg Fe/mL [15]. Other authors demonstrated that nanoflowers had a higher level of internalization than single-core NPs [16]. As a result, high aspect ratio conditions are relevant to how the shape affects NP toxicity. It is also necessary to take into account the various arrangements of shapes and sizes with particular nanomaterial properties.

Area of the Surface and Surface Energy

Since NPs have more surface area and energy, their thermodynamic stability is drastically reduced, which hinders their degree of uniformity, shape, and size [17]. Due to the disruption of the crystal

periodicity, which results in a violation of the electro-neutrality between anions and cations and, as a result, surface change, the surface of crystal oxide NPs is composed of oxygen atoms with a lower coordination number [18]. The proportion of surface-located atoms relative to the total number of atoms rises as NPs get smaller. Because of their small size, numerous active sites, and distinct shape, NPs have a high surface-to-volume ratio. These properties can be used to control reactivity. In comparison to larger particles, the particles with a larger surface area interact with the cell more frequently [19].

Crystallization

The release of metal ions is the most frequent reason why NPs interact toxically with cells [8]. Crystallinity, crystal phase, surface strain, size, defects, media composition, and other factors can affect how something dissolves. Due to the higher percentage of atoms at the corners and edges of NPs, ions from the surface can separate from NPs with less difficulty [20]. For instance, the dissolution kinetics of polar (0001) terminated ZnO NPs in pure water were higher than those of non-polar (1010) crystal surfaces due to the variation in surface Gibbs free energy of different faces [21]. In this regard, He et al. [22] discovered that (1010) surfaces had an oxygen vacancy abundance 3 times higher in the bulk than (000-1) faces.

While pure anatase NPs were more soluble than mixed anatase-rutile NPs, nano-TiO2 containing amorphous phase was more soluble than crystalline TiO2 [23]. Similar to this, nano-anatase demonstrated greater solubility than nano-rutile in artificial media at neutral (pH 7) and low (pH 1.5) pH levels [24]. However, Gurr et al. [25] found that anatase TiO2 NPs of the same size were non-toxic while rutile type TiO2 NPs caused DNA damage, chromosome segregation, and membrane changes.

Aggregation and Dispersibility

When used at a concentration of 1000 ppm, MONPs also exhibit a strong tendency to aggregate due to the Vander Waals forces, higher surface energy, and/or magnetic attraction [26]. The level of aggregation affects the biological and biomedical activities of nanooxides, as well as their biodistribution. For instance, Lousinian et al. [27] found that the inverse relationship between aggregate size and surface area caused the small aggregate size of ZnO NPs to cause higher fibrinogen adsorption than the larger aggregate size. Additionally, it was determined that aggregates of MgO NPs as small as 5 nm could lessen the effectiveness of their interaction with bacteria [28]. As a result, the surface charge of the entire system at various pH levels is determined by the organic or inorganic compounds that stabilize

the engineered NPs in colloidal suspension, such as carbonate, cysteine, or surfactants. When MONPs have not properly stabilized, the colloid instability may cause the particles to aggregate, which could impede or slow their clearance and eventually result in toxicity [29].

Surface Characteristics

Because of their electrostatic attraction to the negatively charged cell membrane, positively charged nanoparticles are thought to be more toxic than negatively charged ones. [30] Their interactions with antibodies, serum proteins, and other substances may have altered the conformation of the molecules that were adsorbed, changing their activity. Positively charged nanoparticles had higher rates of mineralization in human breast cancer than negatively charged ones. [31]. Higher charged micellar NPs were massively incorporated by macrophage, according to Xiao et al.'s research [32], whereas NPs with a slight negative charge indicate high tumor uptake and low macrophage clearance. Additionally, the zero-point of charge and acidity constant values of MONPs [33] that were closely related to the particle aggregation are used to describe the surface properties of MONPs.

Photocatalytic Activity

The formation of holes trapped in surface defects and electrons localized in small hydrogen-rich regions is the most widely accepted mechanism of photocatalysis. The charge separation reduces the likelihood of hole-electron recombination and increases electrochemical activity, photocatalytic activity, and the antibacterial effect [34]. Water molecules from the air or oxidized solution trap the positive holes, creating powerful reactive oxygen species (ROS) called •OH radicals. The oxygen that is reduced to form superoxide (O2•) radicals, which react with H+ to form peroxide radicals (•OOH) or hydrogen peroxide (H_2O_2), can also be trapped by the electrons in the conduction band [35]. Some MONPs, like ZnO, can produce singlet oxygen, superoxide, and hydroxide radicals, while others can only produce one, two, or no ROS molecules [36] or even inhibit ROS production when H_2O_2 is present, indicating anti-oxidant potential [37].

Chemical Composition

The type of MONPs is found to affect the toxicity of nanoparticles, which may be related to NPs' capacity to produce ROS molecules and release metal ions, as was previously mentioned. For instance, NPs of the same size (around 20 nm) as ZnO, SiO2, TiO2, and Al2O3 showed varying degrees of

toxicity on human fetal lung fibroblasts (HFL1) [38]. MTT analysis revealed that ZnO NPs were more toxic to HFL1 after 48 hours of treatment at concentrations of 0.25, 0.50, 0.75, 1.00, and 1.50 mg mL-1, followed in decreasing order by TiO2, SiO2, and Al2O3. While titania, alumina, ceria, and zirconia showed low to moderate toxicity without indicating a correlation between toxicity and either specific surface area or equivalent spherical diameter, another study showed that copper and zinc oxide NPs appeared to be more toxic to two human pulmonary cell types [39].

Target Cell Type

Different metabolic activity and, consequently, different cell death mechanisms and sensitivity to MONP exposure are frequently displayed by different cell target specificity. SiO2 NPs were found to be more cytotoxic on human monocytes (THP-1) than on human lung epithelial cells (L-132) when the toxic effects of nano SiO2 were compared [40]. The ability of monocytes, but not lung epithelial cells, to engage in phagocytosis may be responsible for the cellular target specificities. A549 cells demonstrated less sensitivity than THP-1 cells when the dose- and time-dependent cytotoxic effects of various MONPs were compared to two different cell types, alveolar (A549) and distinguished monocytes to macrophages (THP-1) cells [39].

REVIEW OF LITERATURE

Nanoparticles for Drug Delivery: A Review of the Literature

The fundamental concept behind targeted drug delivery is the use of a controlled drug delivery system. The increased duration of drug circulation, stimulus-controlled drug release at the target site, improved biocompatibility, and therapeutic aspects are the justifications for using a particular drug delivery system. As drug delivery vehicles, a variety of nanocarriers including hydrogel, micelles, dendrimers, nanoparticles (NPs), liposomes, mesoporous materials, scaffolds, etc. are used. Nanoparticles (NPs) are one of these that are used and researched extensively. Due to their small size, NPs as drug carriers increase the survival rate of medications that would otherwise be excreted from the body. Longer drug retention in the body also results in a reduction in the patient's dosage requirement. Doxorubicin, a well-known anticancer medication, is primarily encapsulated on a nanocarrier [41]. Inorganic nanoparticles (NPs) with great potential for ocular drug delivery include Au, Ag, CeO2, Mesoporous Silica, and Magnetic material-based NPs [42]. It is possible to program NPs to respond to external stimuli like temperature, pH, light, enzymes, and various chemical and biological agents. The most popular of these is pH dependence, which is based on the notion that normal tissue and cancerous tissue have different pHs [43]. The current drug delivery system relies on drugs moving through the body on a regular basis without the crucial navigational skills. This is the rationale behind why researchers favor micro- and nanobots with motion capabilities [44]. The fundamental requirement for the drug-carrying nanovehicles is the ability to navigate and reach the desired location.

Table 1.1 Provides a summary of the nanomaterials currently in use for drug delivery applications, including their method of synthesis, shape, size, drug incorporation, and trigger for drug release.

References	Nanostructures	Method	Drug	Trigger	Application
Blanko et.al [45]	Folic acid-PAMAMG3 andG4NPs	Chemical Method	Doxorubicin, Tetracycline and Tamoxifen	Temperature-responsive	Treatment of cancer
Azadani et.al [46]	Car-PEOz-PLA combined with mPEG−PLA to fabricate carnitine-conjugated polymeric micelles to target the carnitine/organic cation transporter 2(OCTN2)	Two-step reaction and film hydration method to form polymeric micelles (combining carnitine-conjugated poly(2-ethyl-2-oxazoline)-poly(D, L-lactide)withmonomethoxypoly(ethylene-glycol)-poly(D,L-lactide))	Coumarin 6 fluorescent dye	Simulated gastrointestinal fluid and simulated intestinal fluid, BALB/mice	Cytotoxicity to Caco-2 cells, delivery of poorly water-soluble drugs
Basu et.al [47]	5-boronopicolinic acid modified poly(lactide-co-glycolide)-g-polyethylenimine (PLGA-PEI)NPs	Self-Assembly method	Camptothecin	pH (5.5 and 7.4),37°C	MCF-7 and 4T1 breast cancer cells
Santhi et.al [48]	Poly(sarcosine) (PSar) and Polyethylene glycol coated Poly(lactic-co-glycolic acid) (PLGA) NPs	Solvent evaporation and oil in water (o/w) method	Docetaxel	Human physiologicalconditions (pH 7.0;temp~37°C)	HeLa. C2BBe1, U-87MG, NCI–N87, HCT-116, NCI–H929-Luc-Ch-Puro cancer cell lines
Alkhayatt et.al [49]	PLGA NPs with Dioleoyl Phosphatidic acid-coated drugs	A water/oil/water double emulsion solvent evaporation	Regorafenib (REGO) and Cisplatin (PT)	Human physiological conditions (pH 7.2;temp~37°C)	Human lung A549 and ovarian A2780 Cancer cells

Chang et.al [50]	Arginine-based poly(ester urea urethane)NPs	Chemical Method and Lyophilization	Gambogic Acid	Folate conjugated	HCC1143, HCC1806, and HCC1395 cells; triple negative breast cancer therapy
Yadav et.al [51]	Folic acid conjugated chitosan coated PLGANPs	Polyelectrolyte complexation method	Folic acid	Simulated Intestinal Fluid, Simulated Gastric Fluid and Simulated Colonic Fluid	Balb/mice Implanted with Colo-26 cells
Park J.H. et.al [52]	Dexamethasone-peptide conjugates	Classic solid-phase peptide synthesis method	Dexamethasone	Incubation	Endotoxin-induced uveitisin rabbit
Kumar R.S.et.al [53]	Hybrid bovine serum albumin-gum arabicaldehyde nanogels	Inverse mini emulsion method	5-fluorouracil	pH-responsive	MCF-7cells
Dehsari H.S. et.al [54]	AL-GP-NG and HA-GP-NG (carbodiimide coupling between glycyl prednisolone(GP), Natural anionic polysaccharides, Alginic acid(AL) and hyaluronic acid (HA)	Organic solvent evaporation from the conjugate solution	Prednisolone	pH-responsive	Anti-arthritic drug-delivery system

RESEARCH GAP

Medical drug-loaded metal oxide nanomaterials are a topic of active and developing research. Here are some research challenges and gaps that scientists have been addressing or may want to look into further:

1. **Optimal Drug Loading and Release:** It can be difficult to strike the right balance between a drug's ability to load and release, and controlled release. To maximize therapeutic efficacy and reduce side effects, researchers must ascertain the maximum drug loading that can be achieved while ensuring controlled and sustained release profiles.
2. **Controlling drug release kinetics** from metal oxide nanomaterials requires the development of sophisticated techniques. In order to control the release of drugs, this involves creating nanoparticle systems that react to particular stimuli (such as pH, temperature, enzymes, etc.).
3. **Stability and Shelf Life**: It is essential to ensure the stability of metal oxide nanoparticles that have been loaded with drugs during storage and transportation. Strategies to stop drug deterioration and nanoparticle aggregation over time should be the focus of research.
4. **Before clinical translation,** it is crucial to evaluate the biocompatibility and potential toxic effects of drug-loaded metal oxide nanomaterials. To fully comprehend how drug loading affects the biological behavior and toxicity profile of the nanomaterials, in-depth studies are required.
5. **Targeting and Selectivity**: It is difficult to increase the specificity with which drug-loaded metal oxide nanoparticles target particular cells, tissues, or organs. To minimize off-target effects, scientists must create nanoparticles that can selectively accumulate at the desired site of action.

AIM AND OBJECTIVE

AIM

Design development and evaluation of bioactive loaded metal oxide nanomaterials for targeted drug delivery system

OBJECTIVE

The major objectives of this study are:

- To design and develop bioactive loaded metal oxide nanomaterials
- To evaluate the structural, surface morphology, optical parameters of developed bioactive loaded metal oxide nanomaterials
- To evaluate the pharmaceutical parameters of developed bioactive loaded metal oxide nanomaterials
- To formulate and evaluate pharmaceutical dosage form containing developed bioactive loaded metal oxide nanomaterials
- To evaluate the bio-assay of developed and evaluated nano materials for their targeted drug delivery action.

METHODOLOGY:

1. Review of literature
2. Procurement of bioactive/s
3. Physiochemical evaluation of procured material.
4. Drug-metal oxide, drug - excipient compatibility study.
5. Synthesis of metal oxide nanomaterials (nano-particles, and nano-tubes).
6. Evaluation of synthesized metal oxide nanomaterials (nano-particles, and nano-tubes).
7. Drug loading on to evaluated of synthesized metal oxide nanomaterials (nano-particles, and nano-tubes).
8. Formulation and evaluation of pharmaceutical dosage form containing Drug loaded on to evaluated of synthesized metal oxide nanomaterials (nano-particles, and nano-tubes) and comparative study with marketed formulation.
9. Bio assay by in-vitro or in-vivo methods to evaluate the intended pharmacological action of selected formulation.

RESEARCH WORK PLAN:

Sr. No.	Work	Period
1.	Literature survey	6 months
2.	Procurement of API and Excipients	1 month
3.	Physicochemical and compatibility study	1month
4.	Synthesis and evaluation of metal oxide nanomaterials	3 months
5.	Formulation & Evaluation of drug loaded metal oxide nanomaterials	6 months
6.	Formulation & Evaluation of pharmaceutical dosage form containing drug loaded metal oxide nanomaterials	6 months
7.	Bioassay evaluation of formulated product by in-vitro or in-vivo study	6 months
8.	Data compilation, drafting of manuscript and thesis writing	7 months
	Total duration of work	36months

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